

## CLAIMS

1           1.     A process for inhibiting misincorporation of a terminator in a  
2     single base primer extension reaction, comprising the steps of:  
3           providing a product of a nucleic acid synthesis reaction, the product  
4     comprising a nucleic acid template and a quantity of inorganic pyrophosphate;  
5           incubating the product and an inorganic pyrophosphatase under  
6     conditions sufficient to decrease the quantity of pyrophosphate, to yield a  
7     purified reaction product;  
8           combining the purified reaction product, a primer, a terminator having a  
9     detectable label, and a polymerase to form a mixture; and  
10          incubating the mixture under conditions sufficient to extend the primer  
11     by addition of the terminator in a single base primer extension reaction,  
12     wherein decreasing the quantity of inorganic pyrophosphate in the product of a  
13     nucleic acid synthesis reaction inhibits pyrophosphorolysis in the single base  
14     primer extension reaction, so as to inhibit misincorporation of a terminator.

1           2.     The process of claim 1 wherein the nucleic acid synthesis  
2     product further comprises a residual reaction component selected from the  
3     group consisting of: a residual primer and a nucleotide.

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1           3.     The process of claim 2 further comprising the steps of:  
2           adding an enzyme selected from the group consisting of: an  
3     exonuclease, an alkaline phosphatase, and a combination thereof to the nucleic  
4     acid synthesis product; and  
5           incubating the nucleic acid synthesis product and enzyme under  
6     conditions sufficient to degrade the residual reaction component.

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1           4.     The process of claim 2 further comprising the steps of:  
2           adding an enzyme selected from the group consisting of: an  
3     exonuclease, an alkaline phosphatase, and a combination thereof to the purified  
4     reaction product; and

5 incubating the nucleic acid synthesis product and enzyme under  
6 conditions sufficient to degrade the residual reaction component.

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1 5. The process of claim 3 or 4 further comprising the step of:  
2 inactivating the enzyme.

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1 6. The process of claim 1 further comprising the step of  
2 inactivating the inorganic pyrophosphatase.

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1 7. The process of claim 1 wherein the detectable label is a  
2 fluorescent label.

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1 8. The process of claim 1 wherein the detectable label is selected  
2 from the group consisting of: an isotopic moiety, a mass tag, a peptide moiety,  
3 a carbohydrate moiety and a combination thereof.

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1 9. The process of claim 1 further comprising the step of detecting  
2 the detectable label.

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1 10. The process of 9 wherein the step of detecting the label  
2 comprises detection of fluorescence polarization.

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1 11. The process of claim 9 wherein the step of detecting the label  
2 comprises direct fluorescence detection, fluorescence quenching, fluorescence  
3 anisotropy, time resolved fluorescence and fluorescence energy transfer.

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1 12. The process of claim 9 wherein the step of detecting the label  
2 comprises detection selected from the group consisting of: radiation detection,  
3 mass spectrometry, and chromophore detection.

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1           13.    The process of claim 3 or 4 wherein the alkaline phosphatase is  
2   selected from the group consisting of: bacterial alkaline phosphatase, calf  
3   intestinal alkaline phosphatase and a combination thereof.

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1           14.    The process of claim 3 or 4 wherein the alkaline phosphatase is  
2   shrimp alkaline phosphatase.

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1           15.    The process of claim 3 or 4 wherein the exonuclease is selected  
2   from the group consisting of: lambda exonuclease, mung bean exonuclease,  
3   Bal31 exonuclease, T7 exonuclease and a combination thereof.

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1           16.    The process of claim 3 or 4 wherein the exonuclease is  
2   exonuclease I.

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1           17.    The process of claim 3 or 4 wherein the enzyme is a  
2   combination of shrimp alkaline phosphatase and exonuclease I.

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1           18.    The process of claim 1 wherein the polymerase is a  
2   thermostable polymerase having a greater affinity for an acyclo nucleoside  
3   terminator than for a dideoxyterminator.

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1           19.    The process of claim 1 wherein the inorganic pyrophosphatase  
2   is selected from the group consisting of: a mammalian inorganic  
3   pyrophosphatase, a bacterial inorganic pyrophosphatase, a yeast inorganic  
4   pyrophosphatase, and a combination thereof.

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1           20.    The process of claim 1 wherein the inorganic pyrophosphatase  
2   is a thermostable inorganic pyrophosphatase.

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1           21.    The process of claim 1 wherein the steps are performed in a  
2   single reaction container.

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1           22.    The process of claim 1 wherein the primer is included in a  
2 primer array.

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1           23.    The process of claim 1 wherein the terminator is an acyclo  
2 nucleoside terminator.

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1           24.    The process of claim 1 wherein the acyclo nucleoside terminator  
2 comprises a detectable label.

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1           25.    The process of claim 1 wherein the detectable label is a  
2 fluorescent label.

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1           26.    A process for inhibiting misincorporation of a terminator in a  
2 single base primer extension reaction, comprising the steps of:

3           providing a product of a nucleic acid synthesis reaction, the product  
4 comprising a nucleic acid template and a quantity of inorganic pyrophosphate;

5           incubating the product and a pyrophosphate removing enzyme under  
6 conditions sufficient to decrease the quantity of pyrophosphate, to yield a  
7 purified reaction product;

8           combining the purified reaction product, a primer, a terminator having a  
9 detectable label, and a polymerase to form a mixture; and

10          incubating the mixture under conditions sufficient to extend the primer  
11 by addition of the terminator in a single base primer extension reaction,  
12 wherein decreasing the quantity of inorganic pyrophosphate in the product of a  
13 nucleic acid synthesis reaction inhibits pyrophosphorolysis in the single base  
14 primer extension reaction, so as to inhibit misincorporation of a terminator.

1           27.    The process of claim 26 wherein the nucleic acid synthesis  
2 product further comprises a residual reaction component selected from the  
3 group consisting of: a residual primer and a nucleotide.

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1           28.    The process of claim 27 further comprising the steps of:  
2           adding an enzyme selected from the group consisting of: an  
3    exonuclease, an alkaline phosphatase, and a combination thereof to the nucleic  
4    acid synthesis product; and  
5           incubating the nucleic acid synthesis product and enzyme under  
6    conditions sufficient to degrade the residual reaction component.

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1           29.    The process of claim 27 further comprising the steps of:  
2           adding an enzyme selected from the group consisting of: an  
3    exonuclease, an alkaline phosphatase, and a combination thereof to the purified  
4    reaction product; and  
5           incubating the nucleic acid synthesis product and enzyme under  
6    conditions sufficient to degrade the residual reaction component.

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1           30.    The process of claim 26 further comprising the step of  
2    inactivating the inorganic pyrophosphatase.

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1           31.    The process of claim 26 wherein the pyrophosphate removing  
2    enzyme is selected from the group consisting of: a pentosyltransferase, a  
3    phosphotransferase, a nucleotidyl transferase and a carboxylase.

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1           32.    A process for inhibiting misincorporation of a terminator in a  
2    single base primer extension reaction, comprising the steps of:  
3           combining a nucleic acid template, a primer, an inorganic  
4    pyrophosphatase, an acyclo nucleoside terminator, and a polymerase to yield a  
5    mixture substantially free of deoxynucleotide-triphosphates; and  
6           incubating the mixture under conditions sufficient to extend the primer  
7    by addition of the acyclo nucleoside terminator, wherein the pyrophosphatase  
8    inhibits pyrophosphorolysis in the single base primer extension reaction,  
9    thereby reducing misincorporation of a terminator.

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1           33.    The process of claim 32 wherein the polymerase has higher  
2   affinity for an acyclo nucleoside terminator than for a dideoxynucleotide  
3   terminator.

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1           34.    The process of claim 32 wherein the polymerase is a  
2   thermostable polymerase.

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1           35.    The process of claim 32 wherein the primer comprises a 3'  
2   terminal nucleotide complementary to the interrogation site nucleotide.

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1           36.    The process of claim 32 wherein the primer comprises a  
2   nucleotide complementary to the interrogation site and wherein the nucleotide  
3   is 2-10 nucleotides upstream of the 3' terminal nucleotide of the primer.

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1           37.    The process of claim 32 wherein terminator is an acyclo  
2   nucleoside terminator.

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1           38.    The process of claim 32 wherein the acyclo nucleoside  
2   terminator comprises a detectable label.

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1           39.    The process of claim 38 wherein the detectable label is a  
2   fluorescent label.

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1           40.    A composition, comprising:  
2           an inorganic pyrophosphatase;  
3           a residual component removal agent selected from the group consisting  
4   of: an alkaline phosphatase, an exonuclease, and a combination thereof; and  
5           a carrier.

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1           41.    The composition of claim 40 wherein the ratio of enzyme  
2   activity units of residual component removal agent to enzyme activity units of  
3   inorganic pyrophosphatase ranges between 1000:1 – 1:1000.

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1           42.    The composition of claim 40 wherein the ratio of enzyme  
2   activity units of residual component removal agent to enzyme activity units of  
3   inorganic pyrophosphatase ranges between 100:1 – 1:100.

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1           43.    The composition of claim 40 wherein the ratio of enzyme  
2   activity units of residual component removal agent to enzyme activity units of  
3   inorganic pyrophosphatase ranges between 10:1 – 1:10.

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1           44.    The composition of claim 40 wherein the alkaline phosphatase  
2   is selected from the group consisting of: bacterial alkaline phosphatase, calf  
3   intestinal alkaline phosphatase and a combination thereof.

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1           45.    The composition of claim 40 wherein the alkaline phosphatase  
2   is shrimp alkaline phosphatase.

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1           46.    The composition of claim 40 wherein the exonuclease is  
2   selected from the group consisting of: lambda exonuclease, mung bean  
3   exonuclease, Bal31 exonuclease, T7 exonuclease and a combination thereof.

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1           47.    The composition of claim 40 wherein the exonuclease is  
2   exonuclease I.

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1           48.    A composition for use in reducing misincorporation of a  
2   terminator in a single base extension reaction, comprising:

3           an acyclo nucleoside terminator;

4           an inorganic pyrophosphate;

5 a pyrophosphatase; and  
6 a carrier.

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1 49. The composition of claim 48 wherein the acyclo nucleoside  
2 terminator comprises a detectable label.

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1 50. The composition of claim 48 wherein the pyrophosphatase is a  
2 yeast inorganic pyrophosphatase.

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1 51. The composition of claim 48 wherein the pyrophosphatase is  
2 selected from the group consisting of: a bacterial inorganic pyrophosphatase  
3 and a mammalian inorganic pyrophosphatase.

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1 52. A commercial package comprising:  
2 a mixture of an exonuclease, an alkaline phosphatase, an inorganic  
3 pyrophosphatase, and a carrier; and  
4 instructions for use of the mixture in a primer extension reaction.

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1 53. The commercial package of claim 52 wherein the exonuclease is  
2 exonuclease I.

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1 54. The commercial package of claim 52 wherein the alkaline  
2 phosphatase is shrimp alkaline phosphatase.

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1 55. The commercial package of claim 52 wherein the  
2 pyrophosphatase is a yeast pyrophosphatase.

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1 56. The commercial package of claim 52 wherein the  
2 pyrophosphatase is a thermostable pyrophosphatase.

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1           57. The commercial package of claim 52 wherein the  
2 pyrophosphatase is selected from the group consisting of: a bacterial  
3 pyrophosphatase and a mammalian pyrophosphatase.

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1           58. The commercial package of claim 52 wherein the mixture  
2 further comprises an additive selected from the group consisting of: a chelator,  
3 a polyol, a reducing agent, a protease inhibitor, a detergent, and a combination  
4 thereof.

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1           59. The commercial package of claim 52 wherein the carrier is a  
2 buffered solution.

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1           60. Use of an inorganic pyrophosphatase in a process for  
2 identification of an interrogation site by single base extension.

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1           61. A process for determining the identity of a nucleotide at an  
2 interrogation site, essentially as described herein.

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1           62. A composition comprising an inorganic pyrophosphatase,  
2 essentially as described herein.

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1           63. A commercial package comprising an inorganic  
2 pyrophosphatase, essentially as described herein.